

AMERICAN MUSEUM *Novitates*

PUBLISHED BY THE AMERICAN MUSEUM OF NATURAL HISTORY
CENTRAL PARK WEST AT 79TH STREET, NEW YORK, N.Y. 10024
Number 3055, 13 pp., 4 figures, 2 tables
March 23, 1993

Missing Link Found: The Second Ancestor of *Gymnophthalmus underwoodi* (Squamata: Teiidae), A South American Unisexual Lizard of Hybrid Origin

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ABSTRACT

A new series of specimens of *Gymnophthalmus cryptus* from Territorio Federal Amazonas, Venezuela, was karyotyped and analyzed by protein electrophoresis. All data available on this bisexual species (distribution, ecology, external morphology, karyotype, and structural genes at 32 loci) indicate that it is the missing ancestor of *Gymnophthalmus underwoodi*. Nearly all of the char-

acters of *G. cryptus* were predicted correctly in previous analyses of *G. underwoodi*. The unisexual and clonal *G. underwoodi*, which has fixed heterozygosity at 42% of its gene loci, originated from one or more hybrids of *G. cryptus* × *G. speciosus* in the Guiana Region of northern South America, perhaps in Venezuela.

INTRODUCTION

Gymnophthalmus underwoodi Grant is an all-female species that occurs in the southern West Indies (Thomas, 1965) and northern South America (Hoogmoed, 1973). Females of *G. underwoodi* reproduce independently by parthenogenesis in the absence of sperm

(Hardy et al., 1989), and inheritance is clonal (Cole et al., 1990).

As with other clones of unisexual lizards (for reviews, see Cole, 1975; Darevsky et al., 1985; Dessauer and Cole, 1989; Vrijenhoek et al., 1989), genetic analyses revealed that

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G. underwoodi had a hybrid origin (Cole et al., 1989, 1990). Its diploid karyotype consists of one haploid complement of chromosomes that are indistinguishable from those of *Gymnophthalmus speciosus* (Hallowell), a diploid bisexual species that also occurs in northern South America. The second haploid set of chromosomes in *G. underwoodi* was unique, not associated with a known bisexual species.

Similarly, protein electrophoresis revealed that the alleles at 33 structural gene loci of *G. underwoodi* included one haploid genome indistinguishable from that of *G. speciosus* plus a second unique genome that was not associated with a known bisexual species. Therefore, an unknown bisexual species of *Gymnophthalmus*, the distinctive karyotype and relevant protein mobilities of which were predicted, was hypothesized to exist in the Guiana Region of northern South America (Cole et al., 1989, 1990). As *G. underwoodi* and *G. speciosus* are morphologically and ecologically similar to each other, the hypothesized species was predicted to be similar in these respects also.

A new species, *Gymnophthalmus cryptus* Hoogmoed, Cole and Ayarzagüena, 1992, was recently described on the basis of preserved specimens. This bisexual species from the Rio Orinoco drainage of Venezuela is morphologically very similar to both *G. underwoodi* and *G. speciosus* and considered as "one of the best candidates for future genetic research to determine whether it is the missing ancestor of *G. underwoodi*" (Hoogmoed et al., 1992: 1). However, no living examples or frozen tissue samples of *G. cryptus* were available for genetic analyses.

Independently of the work described above, one of us (ALM) recently collected five specimens of *Gymnophthalmus* at the following locality: Venezuela: Territorio Federal Amazonas; 5 to 10 km ESE San Juan de Manapiare (5°18'N, 66°03'W). This fieldwork was conducted in conjunction with the Museo de Ciencias Naturales, Guanare, Estado Portuguesa, Venezuela, and involved investigation of the herpetofaunal and ichthyofaunal diversity of Territorio Federal Amazonas.

Realizing that these lizards might represent the hypothesized missing ancestor of *G. underwoodi*, we transported them alive to the

laboratory for karyotypic and biochemical comparisons with *G. underwoodi* and *G. speciosus*. In addition, their morphological characters were compared with those of all other populations of *Gymnophthalmus* in the Guiana Region.

The following questions are addressed in this report: (1) Are the specimens of *Gymnophthalmus* from the vicinity of San Juan de Manapiare *Gymnophthalmus cryptus*? (2) Does *G. cryptus* have the karyotype predicted for the missing ancestor of *G. underwoodi*? (3) Does *G. cryptus* have the structural genes predicted for the missing ancestor of *G. underwoodi*?

ACKNOWLEDGMENTS

ALM's collection of *Gymnophthalmus cryptus* was made possible by the generous cooperation and assistance of several people in Venezuela. Permits were kindly issued by the PROFAUNA section of the Ministerio del Ambiente y de los Recursos Naturales Renovables (MARNR), Caracas, Venezuela. Donald C. Taphorn, Museo de Ciencias Naturales, Universidad Nacional de los Llanos Occidentales Ezequiel Zamora (UNELLEZ), Guanare, Estado Portuguesa, provided invaluable assistance in every way possible. Jaime E. Thomerson of Southern Illinois University, Edwardsville, participated in the expedition to Territorio Federal Amazonas and provided invaluable logistical assistance as well as provocative conversations about the Neotropical biota. The field trip to Amazonas was funded by grants to Dr. Thomerson and Dr. Taphorn from the U.S. National Science Foundation (grant no. 8901678) and the Venezuelan CONICIT (grant no. SI-1978).

Hector Perez and other Piaroa people of the Rio Manapiare served as guides, and their knowledge about the local biota was invaluable in the field. We have much to learn from such indigenous people about biodiversity and the structure and function of ecosystems.

The specimen of *Gymnophthalmus speciosus* analyzed in this report resulted from fieldwork by CJC and Carol R. Townsend in the Republic of Trinidad and Tobago, for which permits were issued by Bheesham Ramdial and Bal Ramdial (Wildlife Section, Forestry

Division). Hans E. A. Boos and John Seyjagat, both of the Emperor Valley Zoo, Port-of-Spain, Trinidad, provided considerable assistance in the field, and Hans, in particular, made the collection of this specimen possible.

The specimens of *Gymnophthalmus underwoodi* analyzed in this report resulted from fieldwork by CJC and Carol R. Townsend in Suriname, for which permits and considerable assistance with logistics in the field were provided by S. A. J. Malone, F. L. J. Baal, M. H. S. Miskin, and H. A. Reichart. Success with the laboratory colonies resulted from the conscientious care provided by Margaret G. Arnold, Colleen C. Coogan, Philip Damiani, Mary E. Holden, Carol R. Townsend, and Anthony Zammit.

In addition to the ways cited above, Carol R. Townsend assisted with the care, karyotyping, and tissue preparations of all of the *Gymnophthalmus* discussed in this paper. We also thank her, Angelo P. Capparella, Darrel R. Frost, and Laurence M. Hardy for reviews and thoughtful comments on the manuscript. Fieldwork by CJC and Ms. Townsend was supported by the National Geographic Society (grants 2198-80, 2622-83, and 3163-85), and laboratory work, in part, by the National Science Foundation (BSR-8105454).

METHODS

EXTERNAL MORPHOLOGY

Color notes and photographs were taken from living lizards. External morphological characters were recorded primarily as described by Hoogmoed (1973), but the number of infralabial scales to the posterior edge of the eye, number of gular scales, number of ventral scales, number of subdigital lamellae, and sex were determined as in Cole et al. (1990; also as in the original description of *G. cryptus* by Hoogmoed et al., 1992).

KARYOTYPES

Chromosomes from vertebral bone marrow and spleen were prepared and studied as described by Cole et al. (1990), but we also used testes in this study. We examined chromosomes of 36 mitotic and meiotic cells from two males of *G. cryptus* (AMNH 138374–138375).

BIOCHEMICAL GENETICS

Frozen tissues were prepared, stored, and analyzed by protein electrophoresis basically as described by Dessauer *in* Cole et al. (1990). For three specimens of *G. cryptus* (AMNH 138374–138376) and three of *G. underwoodi* (FT 2221–2223), tissues were sampled as follows: sex was determined by examining the gonads after slitting the midventral line from the pelvis to the sternum; the pancreas was removed and discarded; the skin was peeled from the body between the arms and legs; the whole torso between the arms and legs (including vertebrae, ribs, musculature, soft internal organs) was removed, frozen (-70°C), and later homogenized; and the voucher was preserved as a unit (head, neck, arms, attached body skin, legs, tail). A fourth *G. cryptus*, FT 2217, was so small (snout-vent length 23 mm) that the whole animal was frozen without a voucher.

Comparisons were made by analyzing together on each gel samples of the four *G. cryptus*, three *G. underwoodi* (from the same laboratory-produced clone studied by Cole et al., 1990), and one *G. speciosus* (from the same population studied by Cole et al., 1990). Thus, cross-correlations were made with the electrophoretic data presented by Cole et al. (1990), and their predictions could be tested directly. Table 1 lists the loci examined, enzyme commission numbers, buffers used, and abbreviations recommended by Shaklee et al. (1989) and/or Murphy et al. (1990).

SPECIMENS EXAMINED

The nine individuals of *Gymnophthalmus* specified below are additional to the nearly 500 specimens examined earlier (Cole et al., 1990; Hoogmoed et al., 1992). Individuals are cited by their unique catalog numbers in the following collections: AMNH (American Museum of Natural History), FT (frozen tissue collection from AMNH at Louisiana State University Museum of Natural Science, Baton Rouge), and MCNG (Museo de Ciencias Naturales, Guanare, Estado Portuguesa, Venezuela). The lowercase letters following the catalog numbers specify the kind of data taken from each specimen, as follows: e, external morphology; k, karyotype; p, protein electrophoresis.

TABLE 1
Presumptive Structural Gene Loci Examined

Locus	EC no.	Abbrev. ^a	Buffer ^b
Oxidoreductases			
Alcohol dehydrogenase	1.1.1.1	ADH	TC, 7.5; NAD, EDTA; Mg
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	TC, 7.5
L-Lactate dehydrogenase	1.1.1.27	LDH-1	PC, 6.0
		LDH-2	
Malate dehydrogenase	1.1.1.37	sMDH	PC, 6.0
		mMDH	
Malate enzyme	1.1.1.40	sMEP	TC, 7.5
Isocitrate dehydrogenase	1.1.1.42	sIDH	TC, 7.5; NADP; EDTA; Mg
		mIDH	
Phosphogluconate dehydrogenase	1.1.1.44	PGDH	TC, 7.5; NADP; EDTA; Mg
Superoxide dismutase	1.15.1.1	sSOD	TC, 7.5
		mSOD	
Transferases			
Aspartate aminotransferase	2.6.1.1	sAAT	TC, 7.5
		mAAT	
Creatine kinase	2.7.3.2	CK-1	TC, 7.5
		CK-2	
Adenylate kinase	2.7.4.3	AK	TC, 7.5
Hydrolases			
Esterase D	3.1.1.-	ESTD ^c	TC, 7.5
Acid phosphatase	3.1.3.2	ACP ^d	PC, 6.0
Dipeptidase	3.4.--	PEPA ^e	TC, 7.5
Peptidase-C	3.4.--	PEPC ^f	PC, 6.0
Tripeptidase-E	3.4.--	PEPE ^g	PC, 6.0
Proline dipeptidase	3.4.13.9	PEPD ^h	TC, 7.5
Peptidase-X	3.--	PEPX ⁱ	PC, 6.0
Lyases			
Aconitase hydratase	4.2.1.3	sACOH	TC, 7.5
		mACOH	
Isomerases			
Mannose-6-phosphate	5.3.1.8	MPI	TC, 7.5
Glucose-6-phosphate	5.3.1.9	GPI ^j	TC, 7.5
Phosphoglucomutase	5.4.2.2	PGM	PC, 6.0
Nonenzymic blood proteins			
Albumin		ALB	V, 8.6
Hemoglobin		HB	TC, 7.5
Transferrin		TF	V, 8.6

^a Based on Shaklee et al. (1989); and Murphy et al. (1990); s = cytosolic enzyme; m = mitochondrial enzyme.

^b Buffer components, pH, and additives: C = citric acid; P = disodium hydrogen phosphate; T = tris; V = veronal (barbituric acid).

^c Substrate 4-methylumbelliferyl acetate; inactive with alpha-naphthyl esters.

^d Substrate 4-methylumbelliferyl phosphate.

^e Substrate phenylalanyl.leucine.

^f Substrate lysyl.leucine.

^g Substrate leucyl.glycyl.glycine.

^h Substrate phenylalanyl.proline.

ⁱ Substrate lysyl.leucine; an extra band on the gels, monomorphic across all the species, that appeared with PEPC.

^j GPI has the wrong pH listed in table 1 of Cole et al. (1990: 6); it is correct here.

Gymnophthalmus cryptus: VENEZUELA: Territorio Federal Amazonas; 5 to 10 km ESE San Juan de Manapiare (5°18'N, 66°03'W) (AMNH 138374–138375, e, k, p; AMNH 138376 and FT 2217, e, p; MCNG 1425, e). There is no voucher specimen preserved for FT 2217, as it was so small the whole lizard was frozen for biochemical analyses.

Gymnophthalmus speciosus: REPUBLIC OF TRINIDAD AND TOBAGO: Chacachacare Island (AMNH 128444, e, k, p).

Gymnophthalmus underwoodi: SURINAME: Brokopondo (FT 2221–2223, e, p; F₃ generation laboratory-colony lizards of the same clone and lineage reported by Cole et al., 1990). These FT numbers are individually correlated with the AMNH numbers when the preserved specimens are entered into the permanent AMNH catalog.

RESULTS AND DISCUSSION

Are the Specimens of Gymnophthalmus from the Vicinity of San Juan de Manapiare Gymnophthalmus cryptus?

In all details of external morphology, habitat, and behavior the new specimens from the vicinity of San Juan de Manapiare agree with *Gymnophthalmus cryptus*. The locality also is consistent with the geographic range of *G. cryptus*.

EXTERNAL MORPHOLOGY

The five specimens from the vicinity of San Juan de Manapiare all agree with *G. cryptus* in all of its diagnostic characters, as follows: unregenerated tail grayish-blue (coppery brown at base); dorsal body coppery brown with pale gold dorsolateral stripe, either fading at midbody or continuing (perhaps as a row of spots) to base of tail; sides very dark brown to black; dorsal scales of body and base of unregenerated tail all smooth; frontal separated from internasals by median contact of prefrontals; one supraocular on each side; usually eight supralabials on each side (seven on one of the specimens), five to the posterior edge of the eye (to suture between supralabials number five and six on three of the specimens); four infralabials on each side counted to the posterior edge of the eye; two pairs of enlarged chinshields; 8 to 10 gulars; ventrals

21 in a female, 22 or 23 in two males, 22 in a juvenile of undetermined sex, and undetermined in one; dorsals 32 in a female, 31 in two males, 33 in a juvenile of undetermined sex, and undetermined in one; 15 scales around midbody in four lizards, 13 in a female; two or three very inconspicuous femoral pores on each side in males (pores absent in females).

In addition, these five specimens all agree with *G. cryptus* in all of the following characters compared: only one dorsolateral light stripe on each side; small body size, snout-vent length 20–28 mm (the males of 25 and 28 mm snout-vent length are adults as both had all stages of meiosis occurring in the enlarged testes); contact of prefrontal with loreal separates frontonasal from anterior supraciliary; frontoparietals absent; nostril low (or a bit below center in one lizard) in the undivided nasal; one loreal, one frenocular, one preocular, and one subocular on each side; three postoculars on each side, the lowest one largest; two supraciliaries on each side, the anterior one very large, posterior one minute; three anterior temporals, two posterior temporals on each side; one postmental; one large central preanal scale, three posterior preanals bordering the cloaca, and three (or four) very small laterals on each side; 12 or 13 subdigital lamellae under the third finger (homologously the fourth, but the first finger has been lost in *Gymnophthalmus*); 14 to 17 subdigital lamellae under the fourth toe; dorsal caudal scales smooth at base of unregenerated tail, keeled on posterior two-thirds of tail.

Hoogmoed et al. (1992) described differences between *Gymnophthalmus cryptus* and *Gymnophthalmus leucomystax*, another species recently described and named (Vanzolini and Morato de Carvalho, 1991). Again, our specimens from the vicinity of San Juan de Manapiare all fit *G. cryptus* and similarly differ from *G. leucomystax*. In particular, *G. leucomystax* has 13 scales around midbody instead of 13–15 (usually 15) and *G. leucomystax* has a sharply defined white area on the upper lip instead of a black upper lip (with or without irregular gray smudges). In addition, the specimens of *G. cryptus* from the vicinity of San Juan de Manapiare have an irregular dark gray to black midventral streak on the chin, as illustrated for *G. cryptus*

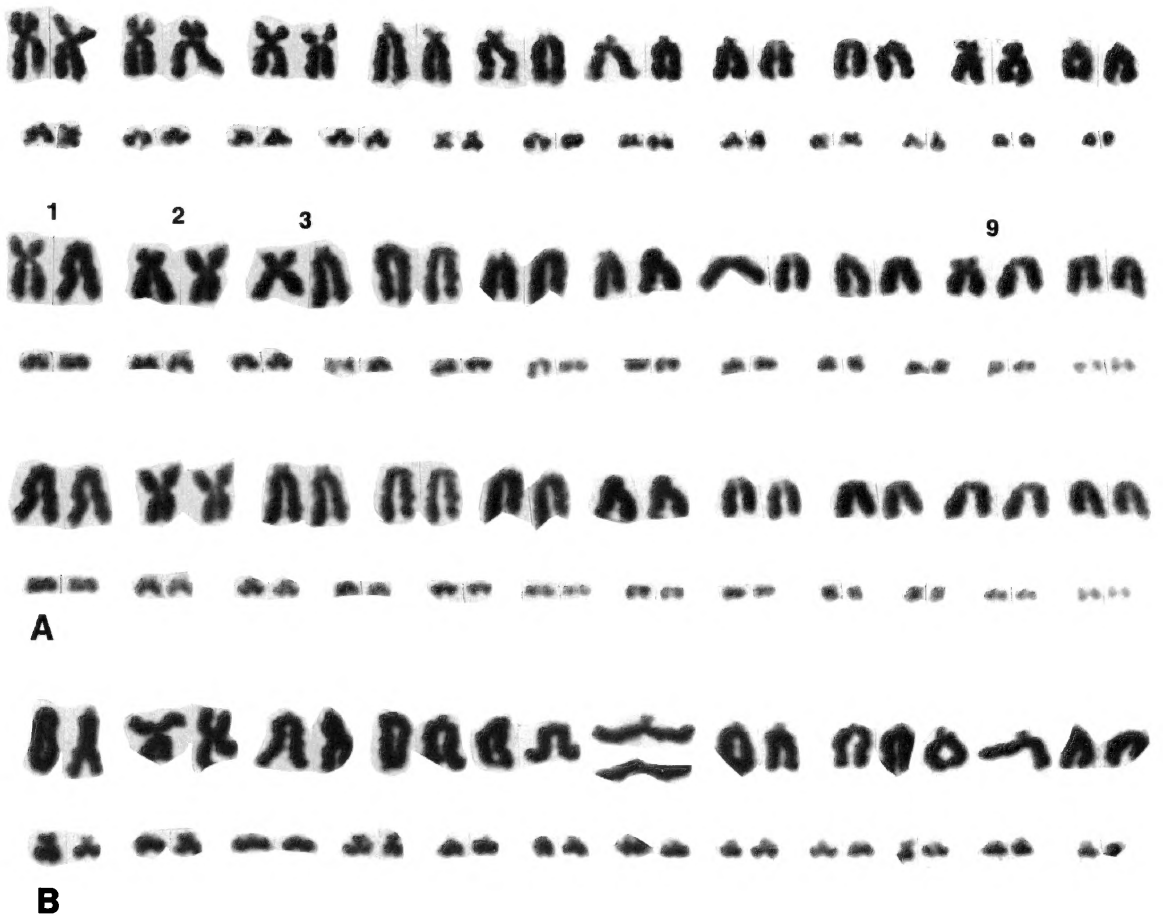


Fig. 1. Diploid karyotypes of three species of *Gymnophthalmus*, each with 44 chromosomes (20 macrochromosomes + 24 microchromosomes). A. This is reprinted from and discussed in Cole et al. (1990: 8; fig. 2). TOP, the bisexual *G. speciosus* (AMNH 128440, male), with pairs 1, 2, 3, and 9 submetacentric or subtelocentric and the remaining macrochromosomes telocentric or essentially so. MIDDLE, the unisexual *G. underwoodi* (AMNH 133314, female), with the left chromosome of each pair matching a haploid set from *G. speciosus*; line represents 10 μ m. BOTTOM, diploid karyotype predicted to occur in the previously unknown but hypothesized missing ancestor of *G. underwoodi*, prepared from extra copies of the right chromosome of each pair of *G. underwoodi* (middle karyotype). B. Actual chromosomes of the bisexual *G. cryptus* (AMNH 138374, male), with pair 2 metacentric and the remaining macrochromosomes telocentric or essentially so.

(Hoogmoed et al., 1992: 5; their fig. 2, middle).

HABITAT AND BEHAVIOR

Hoogmoed et al. (1992) described *G. cryptus* as occurring in leaf litter or around slabs of granite in sunny areas in riverine forest along the Rio Ventuari, at the edge of a road through a forest island in a sandy savanna, at savanna-forest edge, and in areas of forest with clearings, being active from 11:00 to 16:00 hours. The *Gymnophthalmus* reported here were collected in the vicinity of San Juan

de Manapiare, off the floodplains of the Rio Manapiare, a tributary of the Rio Ventuari in the upper Rio Orinoco drainage system. The area is characterized by primary evergreen tropical rainforest and open savanna communities. Individuals were observed and collected at two localities from 7 through 11 August 1991, well into the wet season with high water levels and considerable natural flooding. They were active on a sunny day on the ground alongside and on the lower bricks of an abandoned adobe hut in a small clearing in the forest at 13:00–15:00 hours at air temperatures of 30–31°C. The lizards ap-

peared to be foraging and quickly darted into the shelter of crevices around bricks when approached. They were also found concealed under logs on a rainy day in full-canopied forest, but only a few meters from the water's edge of an open but flooded savanna, at 15:00–17:00 hours, with air temperatures of 28–29°C.

The *Gymnophthalmus* were abundant at these localities, but populations may be spotty in distribution in the area of San Juan de Manapiare. The lizards were not seen in many similar forest edge situations nearby, or deep within forest with a closed canopy. Like other species of *Gymnophthalmus* from the Guiana Region of South America (Cole et al., 1990; Hoogmoed et al., 1992), these appear to prefer sunlight and the shelter of leaf litter and other types of groundcover. Our limited combined observations indicate this species may be more strongly associated with forests than the other species, perhaps suggesting tolerance or requirement of more mesic situations, but also they may shift habitats between wet and dry seasons. Future observations should be directed toward determining habitat preferences during the dry season.

*Does G. cryptus Have the Karyotype
Predicted for the Missing Ancestor
of G. underwoodi?*

Figure 1A, reprinted from Cole et al. (1990; their fig. 2, p. 8), illustrates karyotypes of three species of *Gymnophthalmus*: the bisexual *G. speciosus* (top); the unisexual *G. underwoodi* of hybrid origin (middle); and the karyotype predicted to occur in the second bisexual ancestor of *G. underwoodi* (bottom). Figure 1B illustrates the karyotype we just determined for *G. cryptus*.

G. cryptus has a distinctive karyotype with a diploid number of 44 chromosomes (20 macrochromosomes and 24 microchromosomes). The macrochromosomes are all telocentric, excepting the second largest pair, which is metacentric. Morphology of the microchromosomes is unclear, but as many as seven appeared banded in the clearest cell. Neither secondary constrictions nor morphologically recognizable sex chromosomes were observed in the two males examined. This karyotype (fig. 1B) matches perfectly the one hypothesized to occur in the previously miss-

ing but predicted ancestor of *G. underwoodi* (fig. 1A, bottom).

*Does G. cryptus Have the Structural
Genes Predicted for the Missing
Ancestor of G. underwoodi?*

Proteins encoded by 32 presumptive structural gene loci were compared by electrophoresis in *G. underwoodi* (N = 3 in this study + 67 reported earlier by Cole et al., 1990), *G. speciosus* (N = 1 in this study + 6 reported earlier), and *G. cryptus* (N = 4). As before, the bisexual species showed relatively little heterozygosity and the unisexual *G. underwoodi* showed fixed heterozygosity at a high number of loci (12 of 32 tested for this report, or 38%; table 2, fig. 2).

Considering all of the loci compared for this report (table 2), we identified 44 distinct allele products (referred to hereafter as "alleles," which they reflect) for *G. underwoodi*. Of these, one complete haploid genome of the diploid *G. underwoodi* is identical to a genome that occurs in *G. speciosus*. The one locus (GPI) that appears to be an exception here (table 2) is a polymorphic locus at which the c-allele of *G. underwoodi* is shared with other individuals of *G. speciosus*, as reported earlier (Cole et al., 1990). Combining the results of this study and our last one also suggests that the a-allele at PEPD of *G. underwoodi* was inherited from *G. speciosus*, not the b-allele found in the homozygous state in the most recent individual of *G. speciosus* analyzed (table 2). [In the following discussion we refer to "homozygous loci" and "heterozygous loci," in abbreviated reference to loci with alleles present in those states.]

Considering each locus except one (ESTD, discussed below), the second haploid genome of *G. underwoodi* is identical to a typical genome of *G. cryptus* (table 2). Thus, 43 of the 44 alleles identified in *G. underwoodi* (98%), as well as the complete karyotype, could be found in a typical F₁ hybrid between *G. cryptus* × *G. speciosus*. We conclude that *G. underwoodi* of the northern Guiana Region, Trinidad, and southern West Indies, is a clone of hybrid origin of *G. cryptus* × *G. speciosus* ancestry.

In our previous study (Cole et al., 1990), 33 loci were compared in *G. underwoodi*, *G. speciosus*, and *G. pleei*. The haploid genome

TABLE 2
Alleles^a at 32 Presumptive Structural Gene Loci
in Samples^b of *Gymnophthalmus*

Locus ^c	UND	SPE	CRY	Prediction ^d
ADH	ab	bb	aa	Yes
G3PDH	aa	aa	aa	Yes
LDH-1	aa	aa	aa	Yes
LDH-2	aa	aa	aa	Yes
sMDH	aa	aa	a, b ^e	Yes
mMDH	aa	aa ^f	aa	Yes
sMEP	ab	aa	bb	Yes ^g
sIDH	aa	aa	aa	Yes
mIDH	aa	aa	aa	Yes
PGDH	ac	cc	a, b ^h	Yes
sSOD	aa	aa	aa	Yes
mSOD	aa	aa	aa	Yes
sAAT	ab	aa	bb	Yes
mAAT	aa	aa	aa	Yes
CK-1	aa	aa	aa	Yes
CK-2	aa	aa	aa	Yes
AK	aa	aa	aa	Yes
ESTD	bb	bb	aa	No
ACP	ab	aa	bb	Yes
PEPA	aa	aa	aa	Yes
PEPC	ab	aa ⁱ	b, c ^j	Yes
PEPE	aa	aa	aa	Yes
PEPD	ab	bb	c, b, d	Yes ^k
PEPX	aa	aa	aa	Yes ^g
sACOH	ab	bb	a, b ^l	Yes
mACOH	aa	aa	aa	Yes
MPI	ab	b, a ^m	aa	Yes
GPI	bc	dd	b, a ⁿ	Yes
PGM	ab	b, a	b, a ^o	Yes
ALB	aa	aa	aa	Yes
HB	aa	aa	aa	Yes ^g
TF	ab	bb	a, b ^p	Yes

^a Alleles are designated in alphabetical sequence in order of decreasing anodal migration. As before (Cole et al., 1990), polymorphism was fixed in *G. underwoodi*. For the other species, alleles are listed in order of frequency (highest to lowest) for polymorphic loci.

^b UND = *G. underwoodi*, three specimens of Suriname stock representing the F₃ generation reared in the laboratory. SPE = *G. speciosus*, one specimen from Chacachacare Island, Republic of Trinidad and Tobago. CRY = *G. cryptus*, four specimens from the vicinity of San Juan de Manapiare, Territorio Federal Amazonas, Venezuela. Electrophoretic patterns of these eight specimens were compared with those of the 78 specimens of *Gymnophthalmus* we reported earlier (Cole et al., 1990).

^c For multilocus systems, loci are numbered in order of decreasing anodal migration of their polypeptide products.

^d Yes = The data for CRY fit the prediction of Cole et al. (1990) for the then unknown bisexual ancestor (their ???) that hybridized with *G. speciosus* and gave rise to *G. underwoodi*. No = prediction (Cole et al., 1990) was not met at this locus. Allele designations used here are based on the eight specimens newly examined for this report. Mismatches that can be found by comparing this table with table 2 of Cole et al. (1990: 9) are neither errors nor true inconsistencies, but artifacts resulting from the use of different samples in the different comparisons, particularly having *Gymnophthalmus pleei* and *Arthrosaura kockii* in the samples compared earlier.

^e Frequency of a = 0.62; b = 0.38. The a-allele was predicted for *G. cryptus*.

^f The c-allele at mMDH reported by Cole et al. (1990: 9) for *G. speciosus* (with one dose in *G. underwoodi*) is an error. We checked all samples and confirm here that only the a-allele occurs in all of the *G. underwoodi*, *G. speciosus*, and *G. cryptus* we have examined. The b-allele occurs in *Arthrosaura kockii* and the c-allele in *G. pleei*.

^g The sMEP, PEPX, and HB loci were not tested by Cole et al. (1990), but the results are consistent with the hypothesized hybrid origin and ancestry of *G. underwoodi*.

^h Frequency of a = 0.88; b = 0.12. The a-allele was predicted for *G. cryptus*.

ⁱ Cole et al. (1990: 9) had the relative migration rates at PEPC reversed for *G. speciosus* and *G. ???*. They are correct here.

^j Frequency of b = 0.88; c = 0.12. As corrected above, the b-allele was predicted for *G. cryptus*.

^k PEPD is highly polymorphic in *G. speciosus* and *G. cryptus*. This is the first discovery of the b-allele in *speciosus*, which often (frequency of 0.25; Cole et al., 1990: 9) shares the a-allele with *G. underwoodi*. For *G. cryptus*, frequency of c = 0.62; b = 0.25; d = 0.12. The b-allele was predicted for *G. cryptus*.

^l Frequency of a = 0.62; b = 0.38. The a-allele was predicted for *G. cryptus*.

^m Considering all seven *G. speciosus* we have examined, frequency of b = 0.78; a = 0.22. The a-allele was predicted for *G. cryptus*.

ⁿ GPI is polymorphic in both *G. speciosus* and *G. cryptus*. For *G. cryptus*, frequency of b = 0.88; a = 0.12; both were predicted. If run in a buffer system at pH 6.0, GPI is monomorphic in *G. underwoodi*, *G. speciosus*, and *G. cryptus*.

^o PGM is polymorphic in both *G. speciosus* and *G. cryptus*. For *G. cryptus*, frequency of b = 0.88; a = 0.12; both were predicted.

^p Frequency of a = 0.83; b = 0.17. The a-allele was predicted for *G. cryptus*.

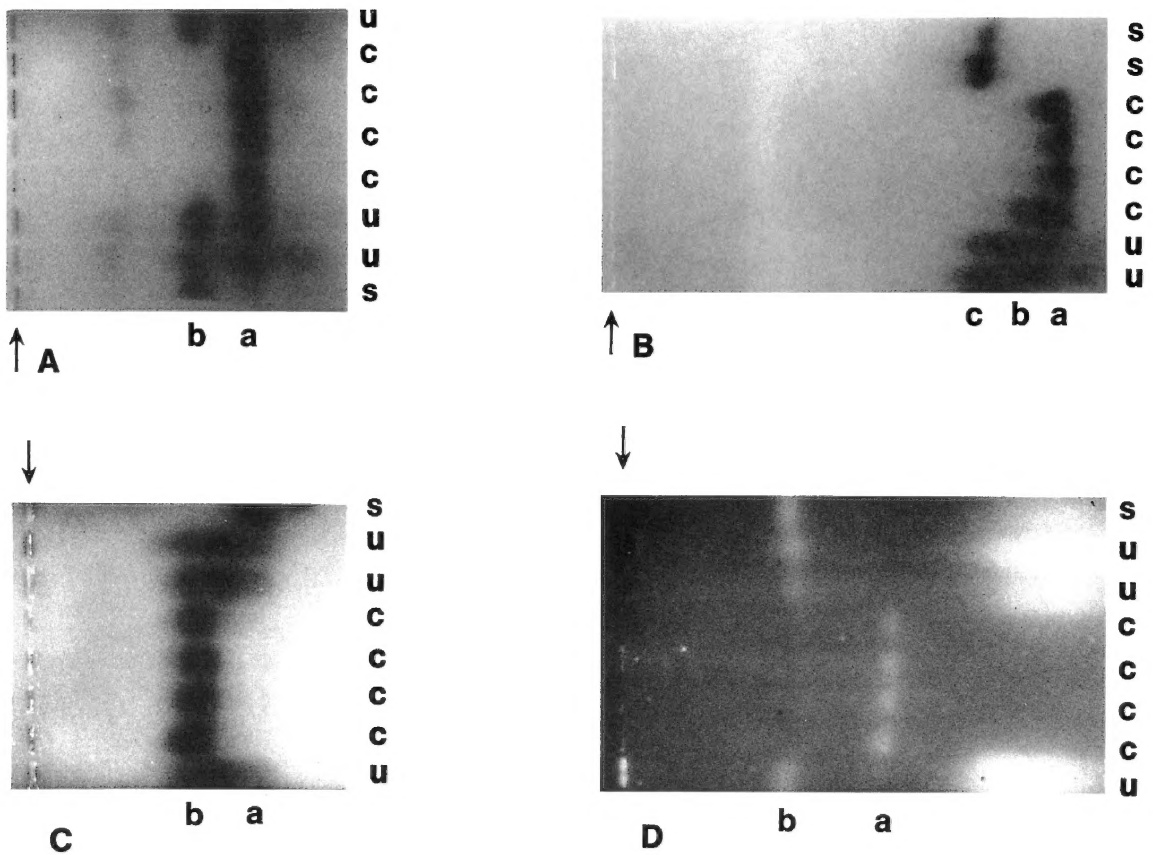


Fig. 2. Electrophoretic patterns of enzymes representing four gene loci (table 2), three of which (A–C) indicate heterozygous genotypes in *Gymnophthalmus underwoodi*, which shares an allele with both *G. cryptus* and *G. speciosus*. A. MPI, eight specimens, *G. underwoodi* heteromorphous. B. PGDH, eight specimens, *G. underwoodi* heteromorphous (one *G. cryptus* heteromorphous also, with the b-allele). C. SAAT, eight specimens, *G. underwoodi* heteromorphous (black half-circle in upper right is an artifact marking the number 1 position on the gel). D. ESTD, the only locus at which *G. cryptus* was unique and did not fit the prediction (table 2), eight specimens. Letters below each gel represent allele designations (table 2). Letters to right of each gel represent species, as follows: c, *G. cryptus*; s, *G. speciosus*; and u, *G. underwoodi*. Anode is to the right; arrows indicate positions of sample applications.

of *G. speciosus* was recognized in *G. underwoodi*, and *G. pleei* was clearly eliminated as the second bisexual ancestor. Fourteen of the 33 loci had alleles in the heterozygous state in *G. underwoodi*, and we predicted that the second ancestor, when discovered, would commonly bear the allele alternative to that of *G. speciosus* at these loci (note correction for mMDH, table 2, fn. f). For the 19 loci homozygous in *G. underwoodi* we predicted that *G. speciosus* and the second ancestor would commonly share the same allele. These predictions have been fulfilled, with only one exception (ESTD).

Twenty-nine of the loci tested were in-

cluded in both of our studies, and the results are rather consistent for *G. underwoodi* and *G. speciosus* (footnotes in table 2 explain the few inconsistencies), while the data for *G. cryptus* fit almost perfectly the alleles predicted for the previously unknown ancestor of *underwoodi*. For these 29 loci (table 2; 11 heterozygous in *G. underwoodi*, 18 homozygous), the data for all but one (96%) are consistent with the hybrid origin of *G. underwoodi* being *G. cryptus* × *G. speciosus*. This includes seven heterozygous loci with alleles predicted to occur in a bisexual species but which had not been found in any other *Gymnophthalmus* except *G. underwoodi* in

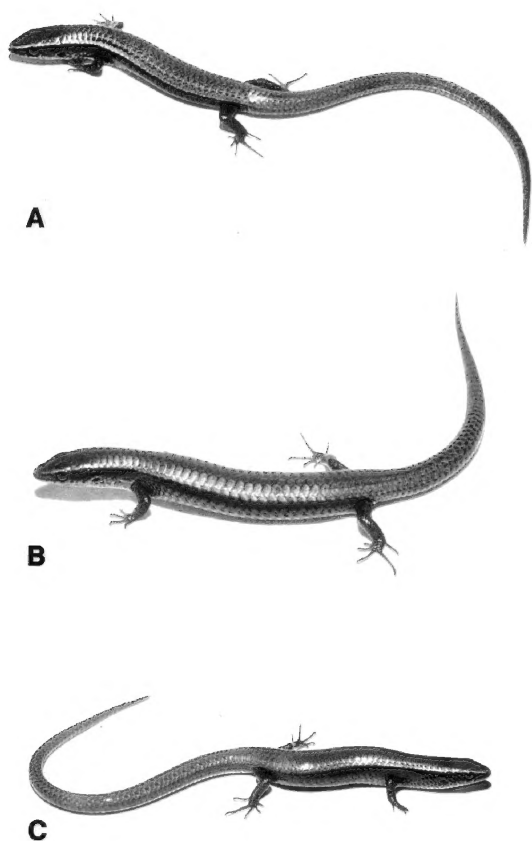


Fig. 3. A. *Gymnophthalmus cryptus* (AMNH 138375; male) from 5 to 10 km ESE San Juan de Manapiare, Territorio Federal Amazonas, Venezuela (snout-vent length, 25 mm). B. *Gymnophthalmus speciosus* (from series of AMNH 128438–128445) from Chacachacare Island, Republic of Trinidad and Tobago. C. *Gymnophthalmus underwoodi*, a unisexual species of hybrid origin (*G. cryptus* × *G. speciosus*); AMNH 119415, female, from Brokopondo, Suriname (snout-vent length, 32 mm). B and C reprinted from Cole et al. (1990: 21; fig. 7).

the past. The only locus that does not fit with the present data is ESTD (table 2), but one mismatch is acceptable, particularly with such a limited sample of *G. cryptus* available ($N = 4$ from one locality). We now predict that the b-allele of ESTD occurs in *G. cryptus* but is yet to be found in this species. Perhaps it occurs in a lower frequency than the a-allele and/or it exhibits geographic variation in frequency. For good examples of geographic variation in allele frequencies in lizards, see Dessauer and Cole (1991).

In addition to the 29 loci included in both of our studies, our last one (Cole et al., 1990) included four loci (ALAT, PK, PEPB, and ADA) that were not included in the present study, and the present study includes three loci (sMEP, PEPX, and HB) that were not included in the earlier one. The three new loci included here (table 2; especially sMEP) are also consistent with the hybrid origin scenario for *G. underwoodi* (*G. cryptus* × *G. speciosus*; fig. 3). To date, 36 loci have been analyzed in *G. underwoodi* and it is heterozygous at 15 of these (42%), making *G. underwoodi* one of the animals with the highest level of heterozygosity known, particularly among diploids for which a substantial sample of loci have been tested (more than 30).

One other study of protein electrophoresis of *Gymnophthalmus underwoodi* has been reported (Martins, 1991), using specimens from populations in Brazil for which we have not had either fresh or frozen tissues. After analyzing 14 loci, Martins (1991) concluded that heterozygosity is low (6–10%) in those *G. underwoodi*, and therefore, he proposed, they may not be of hybrid origin. At first, these conclusions appear to contrast strongly with our results, but further examination shows that they do not. Comparing table 1 of Martins (1991: 694) with our table 1 here, we found that most loci analyzed by Martins are conservative, showing relatively little intra-generic variation in lizards. If we had selected only conservative loci, we might have analyzed only the 20 loci that are homozygous in our sample of *G. underwoodi* (table 2), and we could have erroneously concluded that the species is highly homozygous and not of hybrid origin.

Nine loci may be directly compared between Martins' (1991) study and our present one (his two GOT may be our two AAT; his one IDH may be one of our two IDH; two LDH; two MDH; PGM [although Martins cited a different EC number for it]; and his one SOD may be one of our two SOD). For seven of these nine, we agreed with Martins (1991) in concluding that *G. underwoodi* was homozygous (one AAT; IDH; two LDH; two MDH; and SOD). The remaining two loci are a second AAT and PGM. Using somewhat different experimental procedures, Martins concluded that most individuals of *G. un-*

derwoodi were homozygous at these two loci, whereas we found fixed heterozygosity in *G. underwoodi* at sAAT and PGM (both illustrated by Cole et al., 1990: their fig. 3, p. 10). Although the question remains open whether the unisexual *Gymnophthalmus* from Roraima, Brazil are really *G. underwoodi* (as discussed by Cole et al., 1990; Martins, 1991; Vanzolini and Morato de Carvalho, 1991), it remains premature to conclude that the Brazilian populations are of low heterozygosity, and therefore of nonhybrid origin, until further direct comparisons are made, particularly at the loci reported here as being heterozygous in *G. underwoodi*.

In external morphology, predictions of the characters to be found in *G. cryptus* were not as precise and accurate as were the predictions of the genetic characters (karyotype and genome detected by protein electrophoresis). "We suggest that the second (unknown) ancestor of *G. underwoodi* is similar to both *G. underwoodi* and *G. speciosus* but has, on average, fewer ventrals and dorsals" (Cole et al., 1990: 14). The overall similarity is correct, and females of *G. cryptus* do have an average of slightly fewer ventrals and dorsals than have females of *G. speciosus*, but females of *G. cryptus* and *G. underwoodi* are alike in these characters. In addition, females of *G. cryptus* are most similar to *G. underwoodi* in number of finger lamellae (averaging somewhat more than in females of *G. speciosus*), but they are most similar to females of *G. speciosus* in number of toe lamellae (averaging somewhat fewer than in *G. underwoodi*). *Gymnophthalmus cryptus*, usually with 15 scales around midbody, differs from both *G. speciosus* and *G. underwoodi* (usually 13 or 14). This was not predicted, nor was the very inconspicuous nature of the femoral pores in males of *G. cryptus* predictable.

THE HYBRID ORIGIN(S) OF *GYMNOPHTHALMUS UNDERWOODI* AND BIOGEOGRAPHY

Gymnophthalmus speciosus (with the usual caveats for applying this specific epithet to South American populations; Cole et al., 1990; Vanzolini and Morato de Carvalho, 1991) is broadly distributed in Venezuela, Guyana, and northern Brazil, among other

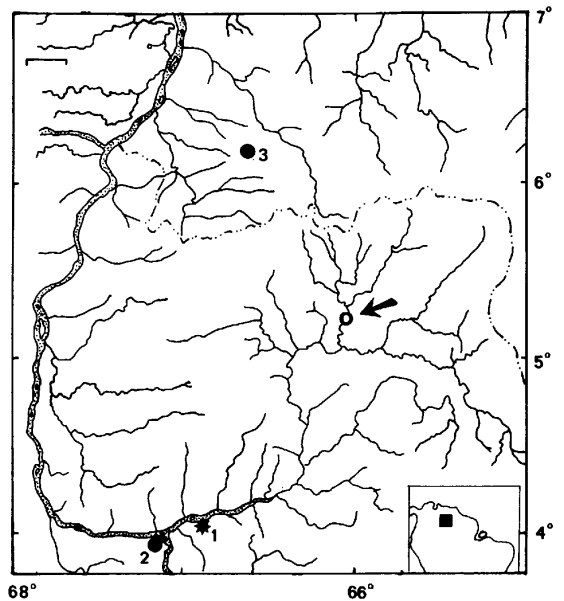


Fig. 4. Map of a portion of western Venezuela and the middle Rio Orinoco drainage system, showing the total known geographic distribution of *Gymnophthalmus cryptus*, slightly modified from Hoogmoed et al. (1992: 2; fig. 1). 1. Type locality, vicinity of Canaripo on the Rio Ventuari, Territorio Federal Amazonas (TFA). 2. Santa Barbara on the Rio Orinoco, TFA. 3. Serrania de Pijiguaos, Estado Bolivar (EB). Open circle at arrow, 5 to 10 km ESE San Juan de Manapiare on the Rio Manapiare, TFA. Inset indicates area of northern South America illustrated in detail; line of dots and dashes separates TFA (to the south) and EB (to the north); and bar for scale in upper left corner represents 25 km.

places, and *G. underwoodi* is known to occur widely in Trinidad, Guyana, Suriname, French Guiana, northern Brazil, and the southern West Indies (see map in Cole et al., 1990: 23). *Gymnophthalmus cryptus* is known only from about four localities in the upper Rio Orinoco drainage in southern Venezuela (Hoogmoed et al., 1992; our fig. 4). At Santa Barbara and to the NNE of Puerto Ayacucho, *G. cryptus* and *G. speciosus* appear to be either literally or nearly sympatric today (Hoogmoed et al., 1992; J. Ayarzagüena, personal commun.). If the hybrid origin of *G. underwoodi* (*G. cryptus* × *G. speciosus*) occurred at or near either of these places (sites 2 and 3 in fig. 4), then *G. underwoodi* probably occurs along the lower Rio Orinoco and elsewhere in eastern (and perhaps southern) Venezuela, although there are no firm records

as yet of *G. underwoodi* for Venezuela. Dispersal of the clone downriver to the north and eastward along the Rio Orinoco would have provided *G. underwoodi* with access to the northern Atlantic Coast of the Guiana Region, Trinidad, and the southern West Indies. Dispersal of the clone upriver to the south and eastward along the Rio Orinoco could have provided access to Roraima, Brazil, although the populations there also could have resulted from southward dispersal through Guyana or eastern Venezuela.

Alternatively, *G. cryptus* may occur more extensively than we realize along the southern edge of the Guyana Shield, and the hybrid origin of *G. underwoodi* could have occurred further to the east, near the convergence of Venezuela, Guyana, and Roraima, with dispersal of the clone radiating from there. Multiple origins, from separate events of hybridization between the same ancestors, may also have occurred. In any event, colonization of the southern West Indies, including the type locality (Barbados), probably followed dispersal of the clone across salt water from the northern Guiana Region (Cole et al., 1990), even if Trinidad may have been colonized when it had a dryland connection to the mainland.

SUMMARY AND CONCLUSIONS

1. The specimens of *Gymnophthalmus* from the vicinity of San Juan de Manapiare, Territorio Federal Amazonas, Venezuela, are *G. cryptus*.

2. *Gymnophthalmus cryptus* is confirmed as being a valid species, even if not morphologically distinct, as its diploid karyotype and genome detected by analyzing 32 loci by protein electrophoresis are considerably different from those of all other congeners.

3. The unisexual *Gymnophthalmus underwoodi* is a clone of hybrid origin, the ancestry of which is *G. cryptus* × *G. speciosus*. *Gymnophthalmus cryptus* was the missing link.

4. The question of whether *G. cryptus* and *G. speciosus* are sympatric at Santa Barbara and other localities can be resolved in the future with karyotyping and protein electrophoresis. Such tests should involve large samples of lizards, as F₁ hybrids and/or *G. underwoodi* may be revealed also.

5. The hybrid origin of *G. underwoodi* occurred at least once in the Guiana Region of northern South America, perhaps in the Rio Orinoco drainage system. The species reached Trinidad and/or the southern West Indies by means of dispersal.

6. The full significance of new collections of *Gymnophthalmus* can be realized only if karyotypic and biochemical analyses are integrated properly with morphological studies. Collectors should be prepared to freeze tissue samples at ultracold temperatures (e.g., in liquid nitrogen) or to transport the living lizards to a site where this is possible.

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